With regard to comment three, red line sketches of figures 8-10 are enclosed with this response. The red line sketches include the item 10.

With regard to comment four, the Brief Description of the Drawings has been amended to obviate the item 25. Support for this amendment is found in figure 8, as well as on page 5, lines 3-19.

Claim Objections

The Examiner states that claims 19-21 are objected to under 37 CFR 1.75(c), as being of improper dependent form. The claims have been amended and are now in proper form.

Claims 5, 9 and 26 are objected to because of various informalities, which have been remedied accordingly. Support for the new and amended claims may be found in the claims as filed.

35 USC §112

The Examiner states that claims 9-12, 22, and 23 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Specifically, claim 9 is rejected as vague over the steps a) and b) of the claim, because the Examiner claims that it is unclear how said target sequence in step a) does not have said binding ligand while target sequence in step b) comprises said binding ligand.

Applicants have amended claim 9 for technical clarity to clarify that after addition of the binding ligand to said target sequence in step a), a target sequence comprising a binding ligand is formed. This amendment merely clarifies step a). Support for this amendment is found in claim 9 as filed.

The Examiner also rejects claim 10, because he suggests that it is unclear how said removing can be done using a double stranded specific moiety.

Applicants respectfully direct the Examiner's attention to the paragraph beginning on page 20, line 27, more specifically, page 21, lines 1-3. Here, Applicants explain that removal accomplished using double-stranded specific moiety methods are based on probe design and content. More specifically, the Specification states that intercalators insert into the stacked basepairs of double stranded nucleic acid. There are also major and minor groove binding

proteins that can distinguish between single stranded and double stranded nucleic acids. Accordingly, these moieties can be attached to a support such as magnetic beads and preferentially bind the hybridized complexes to remove the non-hybridized target probes and target sequences during wash steps. Accordingly, the specification clearly describes how said removing can be done using a double stranded specific moiety.

The Examiner further rejects claim 22 as vague and indefinite over "wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety..." as not being a method.

Applicants have amended claim 22 to clarify that the method includes labeling said target sequence with a functional attachment moiety that binds the support. Support for this amendment is found in claim 22 as filed and throughout the specification, see *e.g.* page 6, lines 10-20.

Finally, the Examiner rejects claim 23, because there is insufficient antecedent basis for the limitation "said support."

Applicants have amended claim 23 to depend from claim 9 and others (see claims). There is sufficient antecedent basis in claim 9 for the limitation "said support." This amendment merely provided antecedent basis for the limitation "said support" hence, support for this amendment is found in claim 9 as filed.

35 USC §102

Claims 5 and 13 are rejected under 35 U.S.C. §102(e) as being anticipated by Barany *et al.* U.S. Patent No. 6,027,889 ("Barany"). Specifically, the Examiner states that Barany teaches the detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions. The Examiner concludes that all limitations of claims 5 and 13 are taught by Barany. Applicants respectfully traverse.

Barany teaches detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions employing a first probe and second probe, a 5' upstream primer-specific portion in the first probe and 3' downstream primer-specific portion in the second probe, Exo I digestion, and basic PCR steps including repeated denaturation, annealing and extension. Figure 15 of Barany describes the allele specific LDR/PCR for

mutations or polymorphisms. Barany teaches allele-specific LDR primers, but does not teach ligation primers with an adapter sequence. Barany also teaches digestion of unligated LDR primers, PCR amplification using zip code primers and finally, detection of fluorescent PCR product.

In contrast, claims 5 and 13 provide method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising the detection position and a second target domain adjacent to the detection position. The method comprises hybridizing a first ligation probe to the first target domain. The first ligation probe comprises an upstream universal priming site (UUP), and a first target-specific sequence. The method further comprises hybridizing a second ligation probe to the second target domain. The second ligation probe comprises a downstream universal priming site (DUP); and a second target-specific sequence comprising a first base at an interrogation position. If the first base is perfectly complementary to the nucleotide at the detection position, a ligation complex is formed. At least one of the first and second ligation probes comprises an adapter sequence. The method further includes removing non-hybridized probes and adding a ligase that ligates the first and second ligation probes to form a ligated probe. The method additionally includes amplifying the ligated probe to generate a plurality of amplicons, contacting the amplicons with an array of capture probes and determining the nucleotide at said detection position. The amplifying is done by hybridizing a first universal primer to the UUP, providing a polymerase and dNTPs such that the first universal primer is extended, hybridizing a second universal primer to the DUP, providing a polymerase and dNTPs such that the second universal primer is extended and repeating these steps.

The law is well established that in order to anticipate a claim, the prior art must disclose "each and every element"of the claimed invention. *SSIH Equipment S.A.v. U.S. Inc. Int'l. Trade Commission*, 218 USPQ 678, 688 (Fed. Cir. 1983). As stated by the Federal Circuit in *In re Bond*, 15 USPQ2d 1566, 1567 (Fed. Cir. 1990), "[f]or a prior art reference to anticipate in terms of 35 U.S.C. § 102, every element of the claimed invention must be identically shown in a single reference.". See also *Glaverbel Societe Anonyme v. Northlake Marketing & Supply, Inc.*, 33 USPQ2d 1496 (Fed. Cir. 1995).

Here, "each and every element" is not present, since Barany does not teach an adapter sequence in the ligation primer, only a zipcode sequence on the PCR primer. That is, the method of Barany includes contacting a target with ligation primers. The ligation primers include priming sites for subsequent amplification. However, the ligation primers do not include an adapter sequence. Following ligation, the ligation products are amplified with PCR primers that include "zip codes" (see figures 15-17).

In contrast, the ligation primers of the present invention contain an upstream universal priming site (on one primer), a DUP (on the other primer) and an adapter sequence (on at least one of the primers). Thus, the ligation primers of the present invention include at least an additional element that is not present in Barany. Accordingly, Barany fails to anticipate the claimed invention.

In addition, the Examiner notes that the "5' upstream primer-specific portion in the first probe and 3' downstream primer-specific portion in the second probe were considered as UUP and DUP or an adapter sequence as recited in claim 5" (under 14. of the Examiner's office action). However, Applicants respectfully submit that this is an impermissible assertion for two reasons. First of all, it appears that the Examiner is reading 2 claim elements of Barany onto 3 elements as claimed. That is, claims 5 and 13 recite 3 elements: a UUP, a DUP and an adapter. Claims 5 and 13 clearly state that the adapter sequence is an independent element of the present invention. Secondly, Barany outlines the use of a "zipcode" component, similar to the "adapter" component in a subsequent step. Thus Barany teaches that the primers are different than a zipcode or an adapter. Hence the Examiner's statement is incorrect.

For the reasons described above, the reference does not anticipate claims 5 and 13, and the rejection is improper. Applicants respectfully request that the Examiner withdraw the rejection.

35 USC §103

Barany *et al.* in view of Walt *et al*

Claims 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Barany *et al.* in view of Walt *et al.*U.S. Patent No. 6,327,410. Specifically, the Examiner states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the

invention to have performed the method recited in claim 5 using an array recited in claims 14-16 in view of the Barany and Walt. The Examiner states that it would have been motivated to modify the method of Barany, because the simple replacement of one kind of nucleic acid array from another kind of nucleic acid array would have been, *prima facie* obvious to one having ordinary skill in the art at the time the invention was made. The Examiner also states that the motivation to make the substitution arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common purpose. The Examiner cites the M.P.E.P. at §§2144.07 and 2144.09 to show support for the obviousness rejection. Applicants respectfully traverse.

Barany has been described above. Again, Barany teaches detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions.

Walt teaches a microsphere based analytic chemistry system and method for making the same. Microspheres or particles carrying bioactive agents may be combined randomly or in an ordered fashion and dispersed on a substrate to form an array while maintaining the ability to identify the location of bioactive agents and particles within the array using an optically interrogatable, optical signature encoding scheme.

In contrast, claims 14-16 provide nucleic acid detection methods using universal priming, as described above, wherein the amplicons are contacted with an array that comprises a substrate with a patterned surface comprising discrete sites, and a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe (claim 14). The discrete sites may comprise wells (claim 15). The substrate may comprise a fiber optic bundle (claim 16). Applicants remind the Examiner that in the method as claimed, the method includes contacting the target with a first ligation probe that includes hybridization and ligation. Adapters are in the ligation primers and not PCR primers, as described above.

When rejecting claims under 35 U.S.C. §103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. See, e.g., *In re Bell* 26 USPQ2d 1529 (Fed. Cir. 1993); M.P.E.P. Section 2142. To establish a *prima facie* case the prior art, either alone or in combination, must teach or suggest every limitation of the rejected claims. Moreover, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece

together the teachings of the prior art to that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1794 (CAFC 1992)

Initially, Applicants respectfully submit that the Examiner has used the claimed invention as a template to piece together the teachings of Walt and Barany. That is, Applicants submit that the Examiner has impermissibly used hindsight reconstruction to create the claimed invention. The Examiner states that "one of ordinary skill in the art would have been motivated to modify the method of Barany, because the simple replacement of one kind of nucleic acid array from another kind would have been *prima facie* obvious." However, Applicants submit that the present invention lays out a blueprint for a possible combination of the two teachings. Before this invention, Barany did not contain any impetus to use the bead based platforms of Walt.

Second, assuming, *arguendo,* that there was motivation to combine the references, the Examiner has still failed to establish a *prima facie* case, because not all elements of claims 14-16 are present in the prior art references. As described above, neither patent contains the element of a ligation probe comprising an adapter sequence. Barany teaches zipcode primers, while Walt is silent with respect to adapters. Because the combination of the references does not contain all elements of the present invention, even if there were motivation to combine the references, the two references would not teach the present invention. As such a rejection under §103 is improper and Applicants respectfully request the Examiner to withdraw the rejection.

Zhang *et al.* in view of Barany *et al.* in further view of Seradyn

Claims 13, 19-23, and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang *et al* U.S. Patent No. 5,876,924 ("Zhang") in view of Barany in further view of Seradyn Particle Technology (November 1996) ("Seradyn"). Applicants respectfully traverse the rejection.

Zhang teaches a nucleic acid amplification method and hybridization signal amplification. The method involves hybridizing a target nucleic acid to several non-overlapping oligonucleotide probes that hybridize to adjacent regions in the target nucleic acid, in the presence of paramagnetic beads coated with a ligand binding moiety. For example, figure 1 depicts the capture, ligation-dependent amplification and detection of a target nucleic acid. Figure 6 shows the amplification and detection of the target nucleic acid employing capture and

amplification probes containing a bound biotin moiety and a single amplification probe. Finally, Zhang does not teach arrays.

Barany has been described above and basically teaches methods of detecting nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions.

Seradyn teaches the use of streptavidin magnetic microparticles to simplify ligand binding. The mechanism utilizes magnetic microparticles as a solid support in immunoassays. Where no magnetic field is applied, particles are in solution. When a magnetic field is applied, the particles are immobilized on the support.

In contrast, claims 13, 19-23, and 26 provide the method of determining the identification of a nucleotide at a detection position in a target sequence described above, wherein the ligation primers contain an upstream universal priming site (on one primer), a DUP (on the other primer) and an adapter sequence (on at least one of the primers). The method may further comprise providing a support on which the target sequence is immobilized. The method may also provide that non-hybridized probes are removed without removing the target sequence from the support. The method may further comprise attaching the target sequence to a support. The method may also provide that the target sequence is attached to the support by a method selected from the group consisting of labeling the target sequence with a functional attachment moiety that binds the support, absorption of the target sequence on a charged support, direct chemical attachment of the target sequence to the support and photocrosslinking the target sequence to the support. The method described above may also implement a support selected from the group consisting of paper, plastic and tubes.

Again, when rejecting claims under 35 U.S.C. §103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. See, e.g., *In re Bell* 26 USPQ2d 1529 (Fed. Cir. 1993); M.P.E.P. Section 2142. To establish a *prima facie* case the prior art, either alone or in combination, must teach or suggest every limitation of the rejected claims. Moreover, it has long been the rule that the claimed invention, as a whole, cannot be said to have been obvious absent some reason or motivation given in the prior art why someone would have been prompted to combine the teachings of the references. *In re Bond*, 15 USPQ2d 1566 (CAFC 1990).

In this regard, Applicants submit that the Examiner has failed to provide any motivation for the combination of the references. While the Examiner claims that it is obvious to combine one LDR/PCR assay for another, it appears that the LDR/PCR reactions of Zhang and Barany are

the same. Thus, Applicants submit that the skilled artisan would not have been motivated to modify either of the references because both references teach the same assay.

Accordingly, Applicants submit that none of the references contain any motivation to combine these references.

In addition, even assuming, *arguendo,* that there was motivation, Applicants submit that the Examiner has failed to establish a *prima facie* case, because not all elements of claims 13, 19-23, and 26 are present in the prior art references. None of the three references contains the element of the ligation probe comprising an adapter sequence. That is, as described above Barany teaches contacting a target with ligation primers. The ligation primers include priming sites for subsequent amplification. However, the ligation primers do not include an adapter sequence. Following ligation, the ligation products are amplified with PCR primers that include "zip codes." However, the claims of the present invention require a DUP, a UUP, and an adapter. Like Barany, Zhang teaches nucleic acid amplification method and hybridization signal amplification employing capture and amplification probes containing a bound biotin moiety and a single amplification probe. Zhang does not add the missing element of the ligation probe comprising an adapter sequence. Likewise, Seradyn does not make up for the deficiencies of Barany and Zhang, because Seradyn teaches the use of streptavidin magnetic microparticles to simplify ligand binding. The mechanism utilizes magnetic microparticles as a solid support in immunoassays. In combination, the above cited references do not teach the claims of the present invention requiring a DUP, a UUP, and an adapter. Accordingly, Applicants submit that not all claim elements are taught in the cited references. As such a rejection under §103 is improper and applicants respectfully request the Examiner to withdraw the rejection.

CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and an early notification of such is solicited. The Examiner is invited to call the undersigned at (415) 781-1989 with any further questions.

Respectfully submitted,
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VERSIONS WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at page 2, line 25, has been amended as follows:

[Figures 1-6 depict preferred embodiments of the invention.]

– Figure 1 depicts a flow chart for array-based detection of gene expression.

Figure 2 depicts a flow chart for array-based detection of RNA alternative splicing.

Figure 3 depicts genome-wide expression profiling using oligo-ligation strategy.

Figure 4 depicts genome-wide RNA alternative splicing monitoring using oligo-ligation strategy.

Figure 5 depicts direct genotyping using a whole-genome oligo-ligation strategy.

Figure 6 depicts whole-genome oligo-ligation strategy.–

The paragraph beginning at page 2, line 26, has been amended as follows:

– Figure 7 depicts a preferred embodiment of the invention utilizing a poly(A)-poly(T) capture to remove unhybridized probes and targets. Target sequense **5** comprising a poly(A) sequence **6** is hybridized to target probe **115** comprising a target specific sequence **70**, an adapter sequence **20**, an upstream universal priming site **25**, an optional label **30**, and a downstream universal priming site **26**. The resulting hybridization complex is contacted with a bead **51** comprising a linker **55** and a poly(T) capture probe **61**.–

The paragraph beginning at page 3, line 22, has been amended as follows:

– Figure 10 depicts a preferred embodiment of the invention, a rolling circle embodiment utilizing a single target probe. Target **5** is hybridized to a target probe **115** comprising a first target specific sequence **15**, detection position **10**, an adapter sequence **20**, a RCA priming site (which may be an upstream universal priming site) **140**, optional label sequence **150** and a second target specific sequence **16**. Following ligation, denaturation, and the addition of the RCA primer and extension by a polymerase, amplicons are generated. An optional restriction endonuclease site is not shown.–

In the Claims

5. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain adjacent to said detection position, wherein said method comprises [comprising said detection position] and a second target domain comprising said detection position[adjacent to said detection position, wherein said method comprises]:

a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence comprising a first base at an interrogation position; and

b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

ii) a second target-specific sequence [comprising a first base at an interrogation position];

wherein if said first base is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed [and] wherein at least one of said first and second ligation probes comprises an adapter sequence;

c) removing non-hybridized [first] probes;

d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

e) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

9. (Amended) A method according to claim 5, 26, 32 or 33 [or 26] wherein said removing comprises:

a) enzymatically adding a binding ligand to said target sequence to form a target sequence comprising said binding ligand;

b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;

c) washing away unhybridized probes; and

d) eluting said probes off said solid support.

10. (Amended) A method according to claim 5, 26, 32 or 33 [or 26] wherein said removing is done using a double-stranded moiety

12. A method according to claim [9] 11 wherein said support is a bead.

13. A method according to claim 5, 26, 32 or 33 [or 26] wherein said amplifying is done by:

a) hybridizing a first universal primer to said UUP;

b) providing a polymerase and dNTPs such that said first universal primer is extended;

c) hybridizing a second universal primer to said DUP;

d) providing a polymerase and dNTPs such that said second universal primer is extended; and

e) repeating steps a) through d).

14. A method according to claim 5, 26, 32 or 33 [or 26] wherein said array comprises:

a) a substrate with a patterned surface comprising discrete sites; and

b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

15. A method according to claim 14 [or 15]wherein said discrete sites comprise wells.

16. A method according to claim 14 [or 15] wherein said substrate comprises a fiber optic bundle.

19. (Amended) A method according to claim 5 or 32,[or 26], further comprising providing a support on which the target sequence is immobilized.

20. (Amended) A method according to claim 19, wherein said non-hybridized [first] probes are removed without removing said target sequence from said support.

21. (Amended) A method according to claim 5 or 32 [or 26], further comprising attaching said target sequence to a support.

22. (Amended) A method according to claim 21, wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety that binds said support, absorption of said target sequence on a charged support, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.

23. (Amended) A method according to claim 9[5 or 26], wherein said support is selected from the group consisting of paper, plastic and tubes.

26. (Amended) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence a first base at an interrogation position; and

c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

ii) a second target-specific sequence [comprising a first base at an interrogation position];

wherein if said first base is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

d) removing non-hybridized [first] probes;

e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

f) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position.

30. (New) A method according to claim 9 wherein said solid support is a bead.

31. (New) A method according to claim 26 wherein said non-hybridized probes are removed without removing said target sequence from said support.

32. (New) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP);

ii) a first target-specific sequence; and

iii) an interrogation position that is complementary to said detection position; and

b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

ii) a second target-specific sequence;

whereby if said interrogation position of said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, wherein at least one of said first and second ligation probes comprises an adapter sequence;

c) removing non-hybridized probes;

d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

e) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

33. (New) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP);

ii) a first target-specific sequence; and

iii) an interrogation position; and

c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

ii) a second target-specific sequence;

whereby if said interrogation position of said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes comprises an adapter sequence;

d) removing non-hybridized probes;

e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

f) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position.

34. (New) A method according to claim 15, wherein said substrate comprises a fiber optic bundle.